THE EMBRYONIC CAPSULES OF NUDIBRANCH MOLLUSCS: LITERATURE REVIEW AND NEW STUDIES ON ALBUMEN AND CAPSULE WALL ULTRASTRUCTURE

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ABSTRACT

Nudibranch egg capsules are small (100-300 μm) transparent structures that surround the eggs inside a gelatinous egg mass. The capsules are produced by the albumen and/or capsule glands of the parent, and usually contain one or more embryos, sperm, and fluid that can contain albumen. In this paper I term albumen any material with a condensed granular ultrastructure observed between the embryonic surface and the inner capsule wall. Although aeolid nudibranchs are said to lack albumen, intracapsular albumen was observed in three species: *Aeolidia papillosa, Coryphella salmonacea,* and *Hermissenda crassicornis*. Preliminary ultracytochemical staining did not detect carbohydrates oxidizable with periodic acid in the intracapsular fluid of 14 day old preveliger *A. papillosa*. Intracapsular fluid from 1, 2, 4, 6, and 7 week old (= ready to hatch) *C. salmonacea* capsules all contained abundant albumen, suggesting that the albumen does not serve a major nutritive role in this species. Treatment of intact *C. salmonacea* capsules with various enzymes did not significantly increase capsule permeability to fixatives and embedding media or increase capsule puncturability. Capsule wall ultrastructure was relatively consistent within each of the six species examined. The capsule walls had no consistent layers and ranged in thickness from 0.07 μm in *H. crassicornis* to 4.5 μm in *Archidoris montereyensis*. Based on data available for the six species examined, capsule wall thickness was not obviously correlated with suborder, developmental type, days to hatching or numbers of embryos per capsule.

Embryos of all nudibranch molluscs develop within tiny, fluid-filled capsules. These capsules average 100-300 μm in diameter and are embedded in gelatinous egg masses (Hurst, 1967; Thompson, 1976). We know little about the formation, structure or adaptive value of either the capsules or the egg masses. The present paper reviews the relevant literature concerning capsule formation, contents, breakdown (at hatching), and adaptive value, and suggests avenues for future research. In addition, this paper presents recent observations on the ultrastructure and fate of the intracapsular albumen, on the ultrastructure of the capsule wall, and on the effect of enzymes on capsule wall permeability.

TERMINOLOGY

The term "capsule," as applied to nudibranch egg masses, is the nonliving spherical to ovoid organic container immediately surrounding the eggs and, as they develop, the embryos (Fig. 1). Therefore, this one structure is sometimes called the egg capsule during early development and the embryonic capsule during later development. Less commonly, this same container has been referred to as the membrane (Ghiselin, 1965), egg sac (Bayne, 1968), egg membrane (Thompson, 1976) or egg-case (Kress, 1971; Thompson, 1976). In giving dimensions of the encapsulated eggs of several opisthobranchs, Rasmussen (1944) occasionally referred to the capsule (diameter) as the uncleaved egg (diameter); he then termed "yolk" what we now call the egg.

In some species, a thin transparent tube called the secondary membrane (Thompson, 1958) surrounds the capsules (= primary membranes). Both of these layers are enclosed by a gelatinous egg mass.

Each capsule contains fluid, which is sometimes referred to in its entirety as albumen. Although albumen, a proteinaceous substance, can occur in this fluid, the fluid itself is more accurately referred to as the intracapsular (= capsular) fluid.

ORIGIN

The capsules are secreted by the female accessory glands of the hermaphroditic reproductive system. This cluster of female glands usually includes a proximal albumen gland and a distal mucous gland, separated by a membrane

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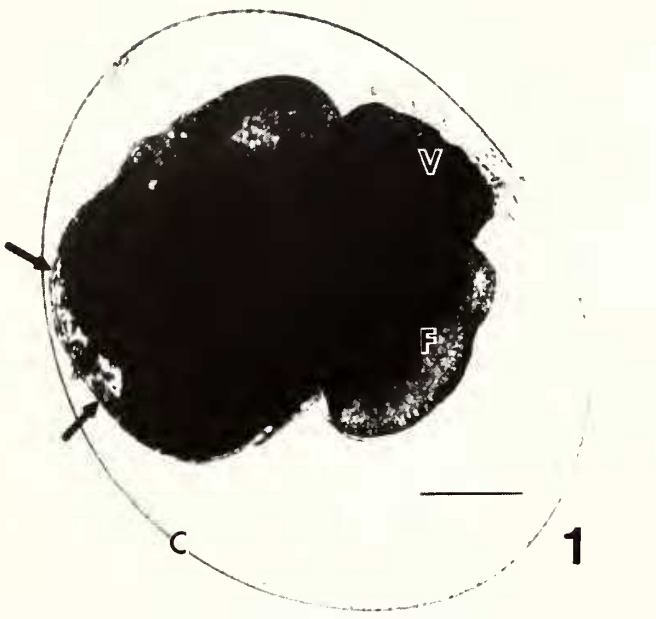


Fig. 1. Light micrograph of nudibranch embryo inside its turgid, fluid-filled capsule (C). The velum (V), foot (F) and part of the shell (arrows) of this six-week old *Coryphella salmonacea* embryo are visible through the transparent capsule wall. Bar = 100 μm.

gland or winding gland (Ghiselin, 1965; Kuzirian, 1973; Thompson, 1976; see complete review for all opisthobranch orders, by Hadfield and Switzer-Dunlap, 1984). During oviposition, a mixture of eggs and allosperm (sperm received during copulation) pass through and are coated by secretions of these glands. The most distal organ, the mucous gland, secretes a gelatinous egg mass that will surround the encapsulated embryos and attach them to the substratum.

The roles of the other more proximal organs are less certain and have rarely been studied. Chambers (1934) examined the reproductive system of *Embletonia fuscata* but could not distinguish the albumen-secreting region of the oviduct from the region that secretes the capsule wall. He referred to the capsule as a "thin but tough 'shell' coat" that is secreted by the "shell gland". However, the capsule of nudibranchs is not a shell and the term shell gland more commonly refers to the invaginated region of the embryonic shell field (see Eyster and Morse, 1984, for review). Lloyd (1952) fixed *Archidoris britannica* during oviposition to examine deposition of the "egg coverings" and concluded that only the intracapsular albumen was deposited by the albumen gland and that the gelatinous layers were produced by the mucus gland; she did not comment specifically on the origin of the capsules. Kuzirian (1973) examined *Coryphella salmonacea* individuals fixed in the act of oviposition and observed a fuzzy layer of 'albumen' (not a capsule) coating the oocytes as they passed through the albumen gland. In contrast, other authors have reported that the albumen gland secretes the capsule wall (Schmekel, 1971; Thompson, 1976); in particular, Schmekel (1971) emphasized that the albumen gland in nudibranchs secretes the capsule wall and "not a layer of protein between egg and capsule." The confusion about which organ secretes which product may occur because the region of the oviduct referred to as the albumen gland by one author may be histologically separable in another species or by a second author into two regions: a proximal area that secretes albumen, and a distal region that secretes the capsule wall. Also, part of this confusion probably arises from retention of the term "albumen gland" in species believed to lack intracapsular albumen (Ghiselin, 1965; Beeman, 1977). More studies of egg capsule deposition are needed to resolve this issue.

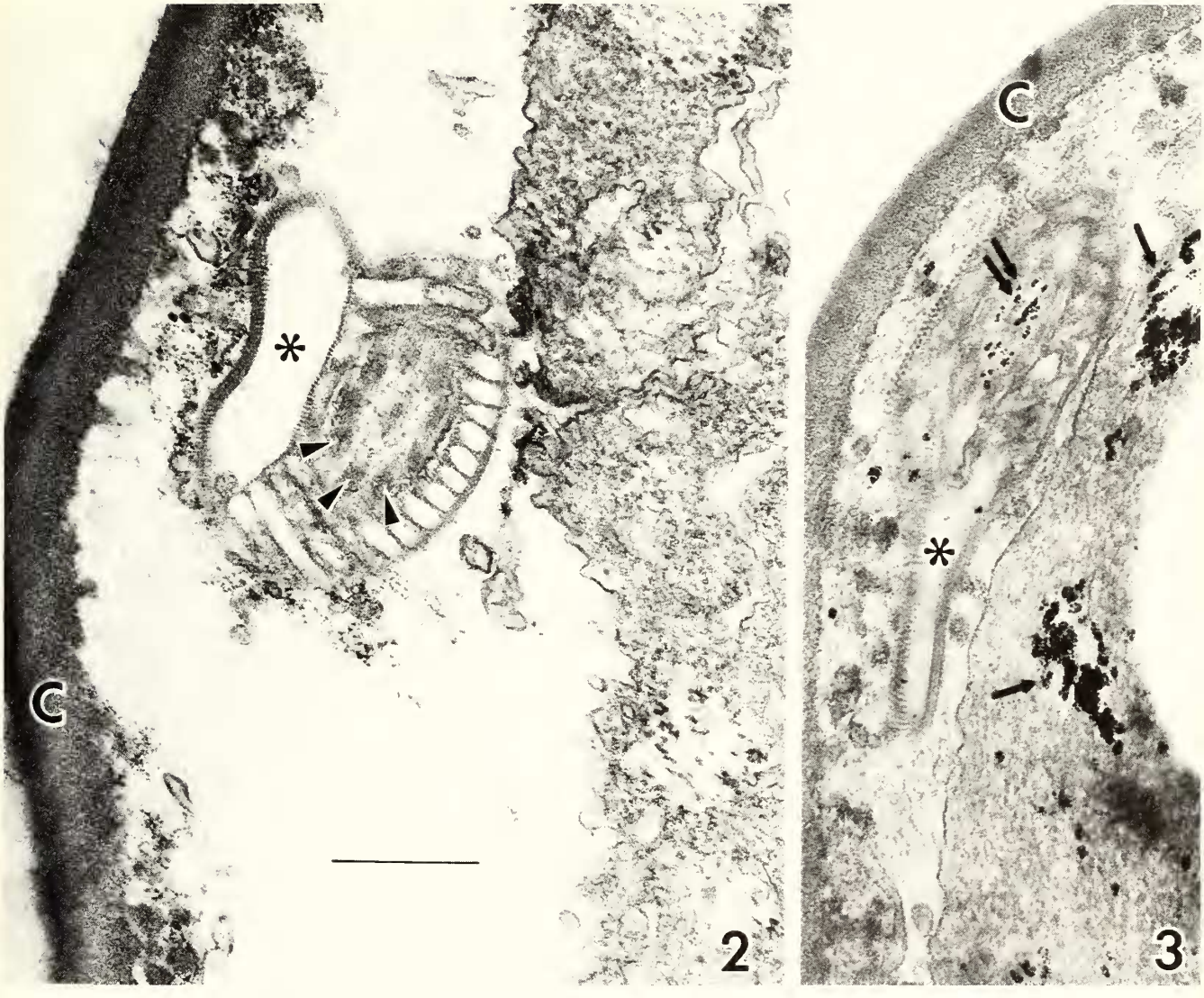
Regardless of the name applied to the organ that secretes the capsules, the egg capsule walls are believed to be formed of neutral mucopolysaccharide in the following manner (based on Ghiselin, 1965). The capsule material is secreted as droplets that will form a thin sheet around the eggs. As the eggs and sheet are rotated by cilia, the sheet surrounds the eggs singly or in groups, depending on the species. Rotation continues and divides the egg covering into packets (individual capsules). Sometimes the locations where a capsule rotated apart from its neighbors are visible as twisted regions of the capsule wall, termed chalazae. The capsule is laid down on the egg (or egg and albumen) surface. The egg is said to then shrink, producing an intracapsular space.

CAPSULE CONTENTS AND POSSIBLE ADAPTIVE VALUE

The gelatinous matrix (= egg mass) surrounding nudibranch egg capsules might protect the developing embryos from infestation, predation, osmotic stress, desiccation stress, mechanical damage, or pollutant stress (Todd, 1981) but the adaptive value of embryonic capsules themselves has not been considered. We can perhaps approach this question by examining the capsule contents. When extruded from the reproductive system of the parent, each capsule typically encloses three things: egg(s), sperm, and intracapsular fluid that may contain albumen. Some capsules lack eggs but whether these capsules also lack sperm and/or albuminous fluid has not been determined. These so-called "empty capsules" are frequently smaller in diameter than egg-containing capsules and are typically located at the beginnings and ends of the egg mass strings or ribbons (Thompson, 1958).

Inside the capsule each fertilized egg either aborts or develops into an embryo. Unlike capsules of some prosobranch gastropods, those of nudibranchs do not serve to enclose nurse eggs; no nudibranchs provide nurse eggs as an extraembryonic food supply. In fact, many species typically have only one egg per capsule (Fig. 1) (Hurst, 1967). In a few nudibranch species, up to 60 eggs can be packaged within one capsule (Hurst, 1967). If an embryo aborts, the capsule physically isolates it from embryos other than capsule-mates; it is unknown if healthy embryos will feed on disintegrating capsule mates.

The capsule remains intact around the embryo for



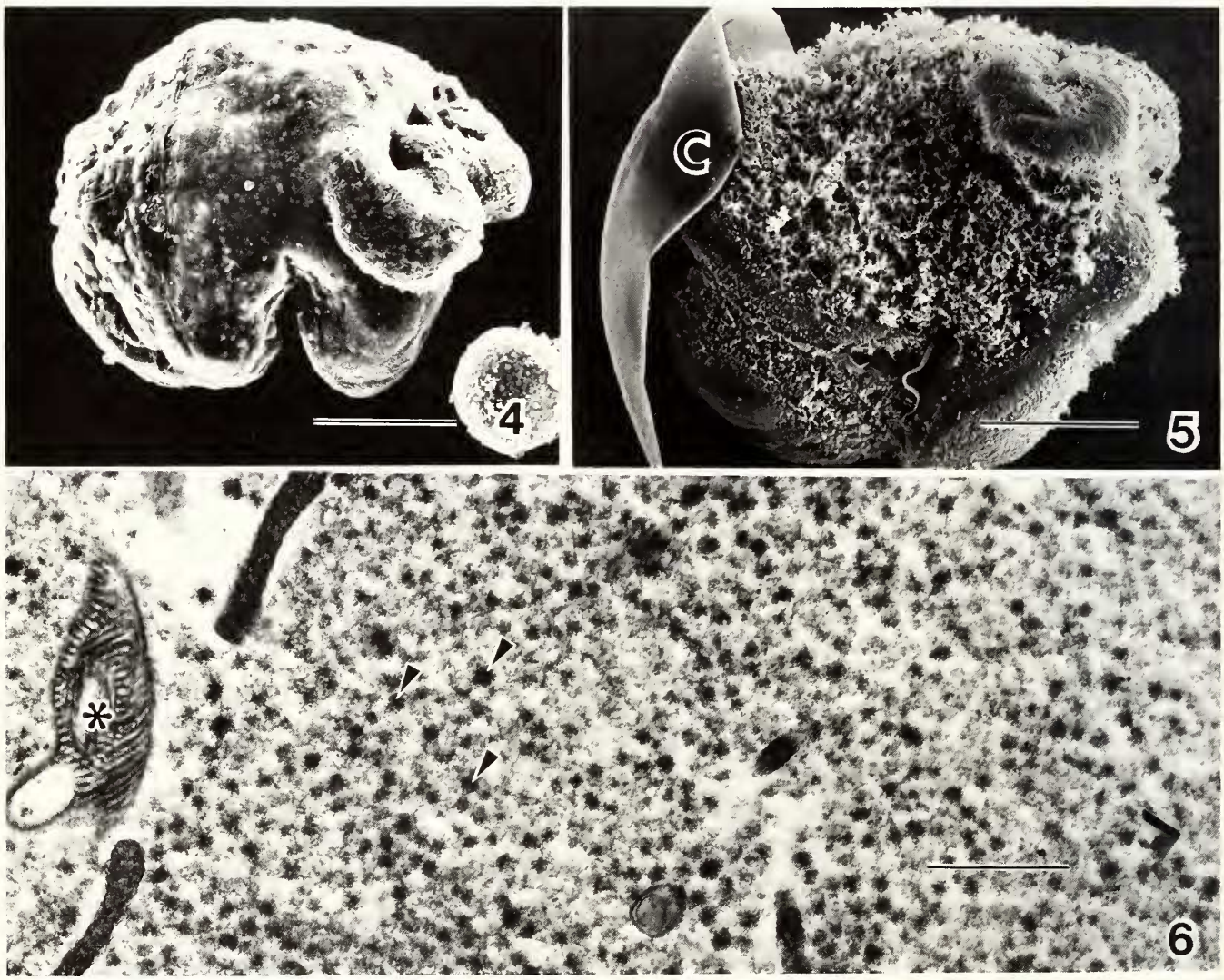
Figs. 2, 3. Transmission electron micrographs of sperm inside *Aeolidia papillosa* capsules (C) 14 days after capsule deposition. The 9 + 2 arrangement of microtubules (arrowheads) is still detectable, as is the periaxonemal sheath and keel (*). Glycogen is not detected in the lumen of the keel (*). **Fig. 2**. Standard TEM preparation followed by staining with uranyl acetate and lead citrate. **Fig. 3**. Standard TEM preparation followed by staining for periodate-reactive carbohydrates (arrows). Bar = 0.2 μm for both.

varying lengths of time from about 1-8 weeks depending on the temperature, the developmental pattern of the species, and various other factors associated with hatching. The organism that hatches from each capsule is either a free-swimming veliger larva or a crawling juvenile, depending on the species. Hatching is discussed below.

In addition to eggs, each capsule encloses multiple sperm (Figs. 2, 3). In nudibranchs, fertilization usually occurs inside the parent soon after gamete mixing (Schemekel, 1971). The fate of the supernumerary sperm is unknown. In some species, such as *Archidoris pseudoargus*, intracapsular sperm are not detected after oviposition, presumably because they are somehow readily degraded (Thompson, 1976). In other species they are visible and are capable of occasional movement several days after oviposition (Thompson, 1976; pers. observ.) In transmission electron microscopy (TEM) sections, sperm are occasionally observed fortuitously (Figs. 2, 3, 6). The sperm were visible with light microscopy within the capsules of *Tritonia hombergi* up to 14 days after oviposition (Thompson, 1976) and were detectable with TEM in *Coryphella salmonacea* capsules 50 days after oviposition (Fig. 6). The energy reserve of the sperm, glycogen-like particles in the helical keel (Anderson and Personne, 1976; Eckelbarger and Eyster, 1981), were not detected in *Aeolidia papillosa* sperm at 14 days (5°C) after oviposition (Figs. 2, 3) or in *Coryphella salmonacea* sperm at 50 days (5-8°C) after oviposition (Fig. 6). In one section subjected to PA-TSC-SP (periodic acid, thiosemicarbazide, silver proteinate) staining

for carbohydrates (Thiéry, 1967; Porter and Rivera, 1979), material associated with the microtubules was periodate reactive (Fig. 3). Little to no periodate reactive substances were detected in the sperm keel (Fig. 3). These observations indicate that the sperm did not decay although their glycogen (energy) supply was apparently exhausted.

The third and last internal component of the capsule is the fluid (and sometimes particulates) lying between the developing embryo and the inner surface of the capsule wall. As the embryo develops cilia, it moves freely within this fluid. In some species the untreated fluid is reported to look granular rather than clear and it is this granular material that is sometimes referred to as albumen. We do not know if untreated albumen is always granular in appearance or how the presence of albumen varies with taxon, development type, or egg diameter. For sacoglossan opisthobranchs, Clark and Jensen (1981) reported three types of albumen: fine granular albumen (< 1 μm diam.), frothy (= alveolar) albumen, and vesicular albumen (up to 10 μm diam., usually attached to inner capsule wall). In the opisthobranch *Phyllaplysia taylori*, Bridges (1972) reported the presence of a large intracapsular body (49 μm diam.) that she believed was food for the embryo. In this paper I will use the term albumen to refer to any condensed, granular material, regardless of its chemical composition, observed with TEM or SEM, between the embryonic surface and the capsule wall.



Figs. 4-6. Electron micrographs of intracapsular albumen in the aeolid nudibranch *Coryphella salmonacea*. **Fig. 4.** SEM of 3½ week old embryo fixed and dried after manual excapsulation. Albumen was washed away from the embryonic surface. Bar = 100 μm. **Fig. 5.** SEM of 7 week old embryo fixed and dried while still inside intact capsule. An obvious layer of flocculent albumen precipitated from the intracapsular fluid is observed on the embryonic surface after the capsule (C) is broken away. Bar = 100 μm. **Fig. 6.** TEM of material lying between surface of 7 week old embryo and inner wall of intact capsule. The abundant granular material (arrowheads) is believed to be albumen. One sperm cross-section is shown at left (*). Bar = 1.0 μm.

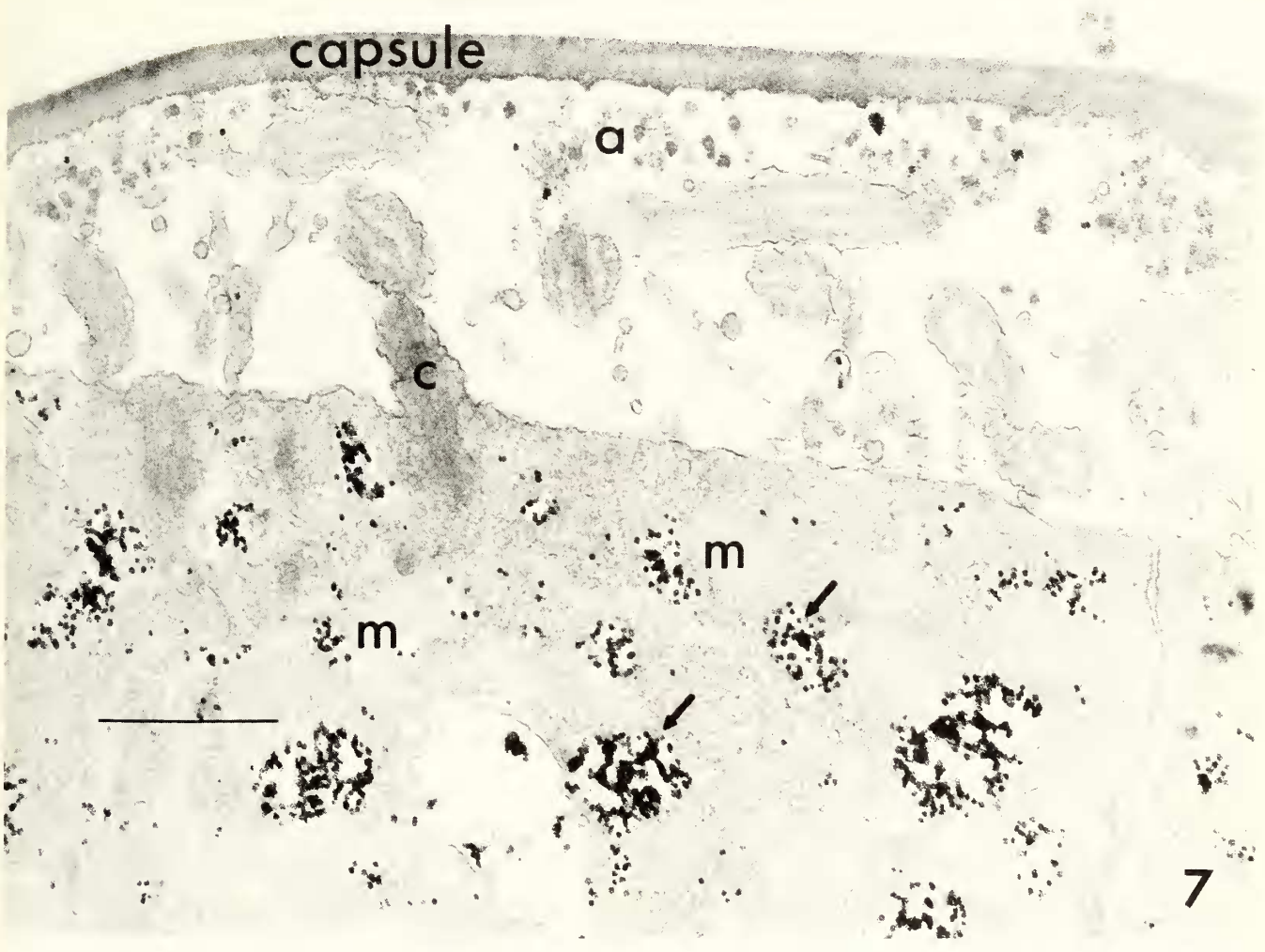


Fig. 7. Transmission electron micrograph of 14 day old *Aeolidia papillosa* (5°C) preveliger embryo in intact capsule. Neither the capsule wall nor the albumen (a) appear to contain carbohydrates oxidizable with periodic acid. The glycogen (arrows), which reacted with the periodic acid, appears electron dense. C = cilium. M = mitochondrion. Bar = 0.5 μm.

Aeolid nudibranchs are said to lack albumen (Ghiselin, 1965; Beeman, 1977). However, Kuzirian (1973) observed "albumen" in three coryphellid species and in the present study a granular substance presumed to be albumen was detected in the intracapsular fluid of three aeolids: *Coryphella salmonacea* (Figs. 4-6), *Aeolidia papillosa* (Fig. 7), and *Hermissenda crassicornis* (Fig. 10). With TEM, the precipitated material appears as electron dense granular material after exposure to glutaraldehyde, osmium tetroxide, uranyl acetate and lead citrate (Fig. 6). Clark *et al.*, (1979) have reported similar condensation upon fixation for albumen of the sacoglossan opisthobranch *Elysia cauze*.

The identification of this presumed albuminous material is not always certain. Although Kuzirian (1973) could detect albumen during passage of oocytes through the oviduct, once the capsule was fully formed, both the albumen and the capsule wall stained so similarly that it was impossible to histochemically distinguish the two with light microscopy. In many TEM sections in the present study it was difficult to ascertain whether some of the observed granular material is part of the movable intracapsular fluid or an integral part of the stationary capsule wall (Fig. 7). In the sacoglossan opisthobranch *Costasiella lilianae* the inner surface of the capsule wall is apparently lined with vesicles that are considered albumen and that break off and are consumed by the growing embryo (Clark and Goetzfried, 1978); the prevalence of this mode of potential embryonic nutrition among opisthobranchs is unknown.

The composition of the intracapsular fluid and particulates of nudibranchs has been examined histochemically by Ghiselin (1965) and Kuzirian (1973). Ghiselin (1965) concluded that albumen was lacking in the aeolid *Hermissenda crassicornis*, and was composed of neutral carbohydrate in the dorid *Dendrodoris albopunctata*. Kuzirian (1973), in contrast, determined that albumen was present in three *Coryphella* (Aeolidacea) species and was a weakly acidic sulfated

mucopolysaccharide. Bayne (1968) histochemically identified both carbohydrate and protein in the intracapsular fluid of the opisthobranch *Aplysia punctata*. In the present study preliminary tests with the PA-TSC-SP stain for periodate-reactive carbohydrates (Thiéry, 1967; Porter and Rivera, 1979) indicated that no carbohydrates oxidizable with periodic acid were detected in the intracapsular fluid of 14 day old (5°C) pre-veliger *Aeolidia papillosa* (Fig. 7). More studies of the chemical composition of the intracapsular fluid may aid our understanding of its possible role or adaptive value.

How might the intracapsular albuminous fluid function? The fluid inside the capsule probably influences diffusional exchange of gases for respiration and of wastes. Although the albumen is often said to be nutritive (e.g. Ghiselin, 1965; Beeman, 1977) there is no convincing evidence that it is. The observation that the perceived granularity sometimes disappears during development is used as evidence that the intracapsular material of nudibranchs is nutritive. However, the granular material may disappear through solubilization rather than through ingestion. Kuzirian (1973) believed that the thin albumen layer observed in capsules of three aeolid nudibranchs served no important nutritional role but rather formed the first mucus layer around the eggs. It would be near impossible to determine the caloric content of the intracapsular fluid from such tiny capsules; the caloric content or dry weight of the capsule and albumen are usually lumped together with that of the intact egg mass (e.g., DeFreese and Clark, 1983; Smith and Sebens, 1983).

To examine the fate of the albuminous material during embryonic development, encapsulated embryos of the aeolid *Coryphella salmonacea* were examined with TEM (by standard techniques; Eyster, 1983) to determine when the albumen disappeared if at all and if there was evidence of albumen uptake by the embryo. All capsules were fixed intact to avoid possible leakage of capsular fluid contents. Intracapsular fluid from 1, 2, 4, 6, and 7 week old capsules (maintained at 5-8°C) all contained abundant albumen. Significantly, albumen was still abundant in capsules from which the young nudibranchs were ready to hatch (Fig. 6). (Hatching readiness was determined by active hatching from adjacent capsules in the same region of the same egg mass). Unless the albumen is consumed immediately upon hatching, this evidence suggests that the albumen does not serve a major nutritive function in this species.

Similar and more detailed studies should be conducted with other species to answer some of the following questions: What is the composition of the intracapsular fluid? Does the composition change during development? Does the albumen ever bind to or derive from the capsule wall? Is any or all of the material ingested? If it is ingested, is it assimilated? Is there evidence of pinocytotic uptake?

For sacoglossan opisthobranchs Clark and Jensen (1981) were able to demonstrate the nutritive importance of albumen by observing prolonged intracapsular development associated with presence of albumen. In another sacoglossan, a different, non-nutritive role has been suggested for the albumen. Chia (1971) suggested that the granular albuminous material inside capsules of the sacoglossan *Acteonia cocksi* was a dehydrated substance serving to expand the capsules via hydration, resulting in increased space for the developing embryos. If this is true for sacoglossans it may also be true for those nudibranchs in which the capsules enlarge as the embryos develop. Kress (1971, 1972) reported that distinct increases in capsular volume occurred in some nudibranch species when the velar cilia developed, perhaps due to uptake or modification of some capsular fluid component or to excretion of wastes. If the albuminous material is to hydrate, it must alter chemically and/or additional water must enter the capsule from outside. This influx of water could follow a change in capsule permeability to water or an increase in internal osmotic concentration. Not all sacoglossans have capsule enlargement (Chia, 1971; Kress, 1971, 1972) and among nudibranchs degree of enlargment varies among species (Kress, 1971, 1972, 1975). A study correlating presence/absence of albumen and capsule enlargement has not been undertaken. It may also be informative to determine if changes in capsule volume are accompanied by changes in capsule fluid histochemistry. If albumen is present in so-called "empty" capsules and if these capsules do not enlarge when neighboring embryo-containing capsules do, we may conclude either that the albumen is not involved in capsule enlargement or that presence of an embryo alters the albumen.

CAPSULE PERMEABILITY

Strathmann and Chaffee (1984) have recently discussed factors that are likely to influence oxygen diffusion through gelatinous egg masses such as those of opisthobranchs; however, the permeability of nudibranch capsules and egg masses to oxygen, water, metabolic wastes, dissolved nutrients, and salts is an unexplored subject. Some preliminary data on capsule permeability and the effects of enzymes on permeability and puncturability are therefore presented below. During a study of *Coryphella salmonacea* embryonic shell formation (Eyster, 1985) I observed that embryos within broken capsules sectioned better than those with intact walls. The intact capsule apparently inhibited passage of fixatives and/or embedding media through the capsule wall. This was true throughout prehatch development, indicating that capsule permeability to the fixative did not increase with age. Because of poor penetration of fixatives and/or embedding media through the capsule wall, I explored methods of removing the capsule from around the embryo or of altering capsule permeability prior to fixation. The egg capsules usually were easily dissected from the gelatinous egg mass in this species. Manual removal of the 350 x 430 μm diameter capsules without damaging the embryos could be accomplished following micropuncture of the capsule wall (see technique in Eyster, 1985) but was a difficult and tedious procedure. As the capsules are probably partly protein and partly carbohydrate (Ghiselin, 1965; Bayne, 1968; Kuzirian, 1973), I tried improving capsule permeability by briefly incubating intact capsules in enzymes (Table 1, including two proteolytic enzymes and three which act on carbohydrates) prior to standard TEM fixation. Capsules were removed from the

Table 1. Enzymes (0.1 mg/ml) used to pretreat intact 15 day old *Coryphella salmonacea* capsules prior to preparation for transmission electron microscopy. (+ = yes; - = no; ± = result inconsistent; blank = not tested) N = 3 or more capsules for each.

Enzyme	Treatment Time (min.)	Improved sectioning quality?	Increased puncturability?
trypsin	15	-	-
	45	-	±
protease	15	-	-
	45		±
α-amylase	2	-	-
	10		-
	20		+[1]
hyaluronidase	1		-
	4		-
	10		-
	30		-
	60	±	±
amyloglucosidase	2		-
	4		-
	6		-
	8	±	±
	10	±	-
	30	±	-
	45	±	

[1]Although capsule puncturability was improved, the enclosed embryo disintegrated.

gelatinous egg mass and were incubated with each enzyme (0.1 mg/ml of seawater) from 2-60 minutes (Table 1). After incubation, some of the enzyme-treated and untreated capsules were prepared for TEM. In all cases, embryos in micropunctured, untreated capsules were better fixed and/or infiltrated than embryos within intact capsules that were enzyme treated up to one hour. Among the pretreatment enzymes, only hyaluronidase and amyloglucosidase produced any sectionable embryos and results varied among capsules within the same test.

Untreated capsules of *C. salmonacea* were too turgid to pinch with forceps or to easily puncture. Enzyme-treated capsules were poked and prodded with forceps and microprobes to determine if the enzyme pretreatment facilitated manual capsule removal. All of the enzymes seemed to alter capsule turgidity (or at least capsule puncturability) but results varied from capsule to capsule (Table 1). In another attempt to decrease the difficulty of manually removing *C. salmonacea* capsules by first decreasing capsule turgidity, I subjected 10 day old, intact embryonic capsules (maintained at 30 ppt) to increased salinities (34, 35, 42, and 76 ppt). The 76 ppt and 42 ppt salinities were prepared with Instant Ocean in distilled water; the other salinities were prepared by adding Instant Ocean to natural 30 ppt seawater. In 76 ppt salinity the capsules soon lost turgidity, and the embryos began to disintegrate within 15 minutes. This presumably reflects outward diffusion of water across the capsule walls from higher internal to lower external water concentration and a corresponding increase in intracapsular osmotic concentration. At 42 and 35 ppt the capsules also lost turgidity but without corresponding disintegration of the embryos. At 35 ppt, capsule turgidity decreased within five minutes, but at 34 ppt about 12 minutes were required before the capsule lost sufficient turgidity (= lost enough water) to be micropunctured. Capsules also lost turgidity and became puncturable for 1-2 minutes when placed in glutaraldehyde fixative (~1200 mosm). However, after a few minutes in the fixative they often unexplainably regained turgidity and could not be readily punctured.

Other data suggest that the capsule wall is also an effective barrier to the calcium chelator EGTA (ethylene-glycol-bis-N,N-tetraacetic acid). Shells of encapsulated veligers of the nudibranch *Dendronotus frondosus* remained birefringent after a 30 min. incubation in 10 mM EGTA, whereas shells of newly hatched veligers began to lose birefringence (= lose shell $CaCO_3$) within 3 min. (Eyster, 1986). Data such as these suggest that the capsule wall is an effective barrier to EGTA.

These preliminary data suggest that the capsule walls of *Coryphella salmonacea* are permeable to water but not readily permeable to larger molecules such as those of salts, fixatives, and embedding media. Since the osmotic concentration apparently increased inside the treated capsules as water moved out, "albumen" probably did not exit through the walls. The ability to retain intracapsular albumen in the face of environmental salinity change may be important to the embryos if albumen contributes to successful development. Clark *et al.* (1979) reported the presence of an extracapsular yolk string that disappears during embryonic development in the sacoglossan *Elysia cauze* and suggested that embryonic enzymes might exit the capsule and dissolve this yolk, which then diffuses into the capsule. Clark has since stated he no longer thinks the yolk can pass into the capsule through the wall (Hadfield and Switzer-Dunlap, 1984).

PREDATION AND CAPSULE CONSUMPTION

Feeding on nudibranch egg capsules and masses is poorly documented. Fish have been observed to ingest nudibranch egg masses but it is not clear that the fish seek the egg masses as a natural food source. In the laboratory, I have observed adult *Coryphella salmonacea* and *Armina tigrina* feeding on their own egg masses, but this may be a sign of hunger rather than of natural dietary preference. There are several opisthobranch species reported to naturally feed on the egg masses of other opisthobranch species (Crane, 1971; Haefelfinger, 1962, cited by Gascoigne and Sigurdson, 1977). Chia (1971) observed that *Acteonia cocksi* (Sacoglossa) fed on their own egg capsules after hatching from them.

HATCHING

Although the method of hatching has not been demonstrated for any nudibranch, possible mechanisms of

capsule rupture/breakdown (resulting in hatching) include enzymatic degradation, osmotic rupture, physical activity of the embryo, and degradation by bacteria and protists (Hurst, 1967; Harris, 1975; Davis, 1981; Todd, 1981). If hatching is a developmentally programmed event, then salinity and temperature will affect onset of hatching by altering rate of embryonic development, but there is no evidence that changes in either of these factors normally stimulate hatching in nudibranchs.

Hatching can be artificially delayed in the laboratory by maintaining egg masses in static culture (no aeration, change of filtered seawater and dishes daily) rather than in flowing seawater (Hurst, 1967; Harris, 1975; Rivest, 1978; Eyster, 1979, 1985). For example, I collected pairs of egg masses laid on the same day in the laboratory by *Aeolidia papillosa*, *Tenellia pallida*, or *Coryphella salmonacea* and divided them between flow-through and static culture conditions. The egg masses placed in flowing seawater hatched before the masses kept in static culture. Embryos in static culture often rotated in their capsules more slowly. If egg masses in static culture were then aerated or transferred to fresh seawater, the young nudibranchs increased their activity rate and soon hatched. These observations suggest several possibilities: 1) Flowing water may provide more oxygen to the developing embryos. In static culture low intracapsular oxygen concentrations may evolve and inhibit development. 2) Flowing water may increase rate of diffusion of embryonic wastes out of the capsules. Waste build-up in static culture may inhibit embryonic development and embryonic activity. 3) Transfer of newly laid egg masses to clean dishes and filtered seawater may decrease abundance on/in egg masses of bacteria, which have been implicated in promoting nudibranch hatching (Harris, 1975). These three possibilities could be tested in the laboratory by controlling water flow, dissolved oxygen levels, and bacterial abundance.

Hatching may involve more than one mechanism. Even if nudibranch embryos do not produce hatching enzymes, the capsule wall may be altered during development in response to increased intracapsular osmotic pressure. As mentioned above, Kress (1971, 1972) has demonstrated that the capsules of some nudibranch species swell during development. Although the capsules may swell during development, they seem to lose their normal turgidity just prior to exit of the embryo and are readily deformable even by the pressure of velar cilia (Thompson, 1958; Perron and Turner, 1977; pers. obs.). Nudibranch capsules do not seem to burst open and then shrink like punctured balloons because the capsule walls are not as elastic. After hatching the capsules are typically flaccid. The hatching mechanism may be different for the antarctic *Austrodoris macmurdensis*, which is reported to have unusual chitin-reinforced capsules that are tightly abutted in a beehive-like arrangement (Gibson, *et al.*, 1970). Hatching was effected through ruptures in the uncollapsed capsule wall.

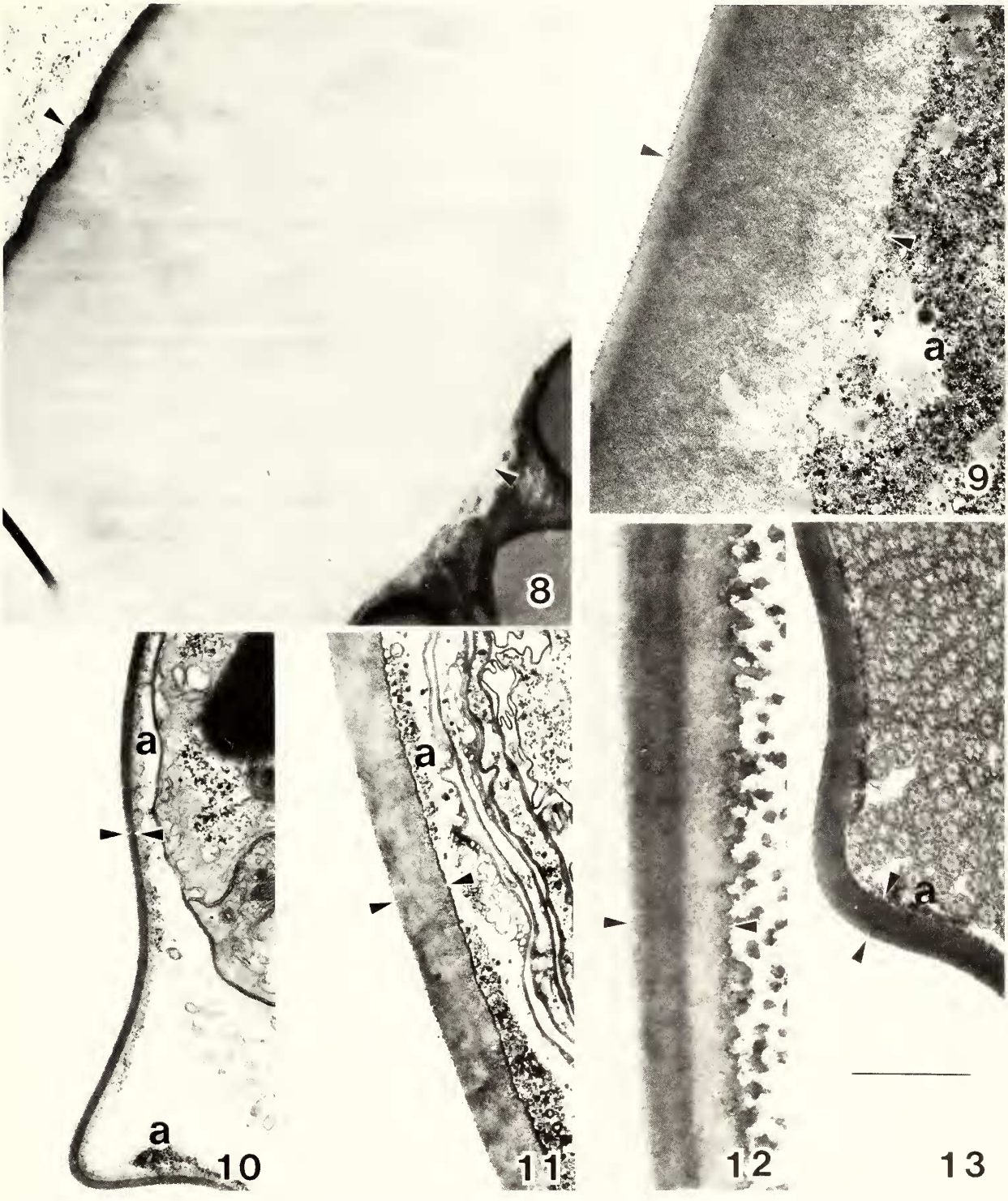
If a capsule increases in diameter during development, it must simultaneously decrease in wall thickness, unless new wall material can be added from the intracapsular fluid/albumen. There is no reason to believe that embryonic secretions are added to the wall and there is no ultrastructural evidence of preformed capsule wall indentations that could allow for capsule expansion. Although neither change in capsule thickness over time nor binding of albumen to the capsule wall have been demonstrated to occur, the former (decreased capsule wall thickness) might ease mechanical or chemical hatching for the embryo, and might provide less of a barrier against bacterial and protozoan invaders. A thinner capsule wall may also be more permeable to oxygen and wastes. Studies of capsule wall structure and permeability from deposition to hatching might provide some clues to how nudibranch embryos hatch.

CAPSULE ULTRASTRUCTURE

Most nudibranch capsules are so thin that transmission electron microscopy is needed to examine their structure. For the present study, capsule ultrastructure is shown for six species (Figs. 7-13). All capsules were obtained from egg masses deposited in the laboratory. Adults were obtained from the following locations: *Archidoris montereyensis* (Friday Harbor, WA); *Cadlina laevis* (Shoals Marine Laboratory, ME); *Hermissenda crassicornis* (courtesy of June Harrigan, Woods Hole, MA, from Californian adults); *Coryphella salmonacea* and *Aeolidia papillosa* (Nahant, MA); *Dendronotus frondosus* (Eastport, ME).

For five of the six species some capsules were fixed when the enclosed embryos were trochophores. Random additional capsules were also fixed. All capsules contained one healthy individual, except for those of *Aeolidia papillosa*, which contained three. For the two dorids, only one egg mass each was available. For *Dendronotus frondosus* capsules from two different egg masses at different stages of development were used (half-shelled veliger stage, fully-shelled veliger stage). For two of the aeolid species, capsules were examined from at least two egg masses from different parents and/or from two stages of development (over time) from the same egg mass. For the third aeolid (*Aeolidia papillosa*) I examined capsules from a single egg mass, fixed at four times over a single day (312, 315, 325, 335 h after oviposition).

For each species the egg mass matrix was teased open and capsules were removed and pipetted into the fixative. Following glutaraldehyde-osmium tetroxide fixation and uranyl acetate—lead citrate staining (Eyster, 1983), the capsule walls of all species examined were at least moderately electron dense and in most species did not exhibit any consistent distinct layers. In the few available sections of *Archidoris montereyensis* capsules the outermost portion ($\sim 0.1\mu$m wide) of the capsule was distinctly more electron dense but not obviously different in texture from the rest of the capsule (Fig. 8). This narrow outer zone of the capsule was as wide as the total capsule wall of *Aeolidia papillosa* (Fig. 7) or of *Hermissenda crassicornis* (Fig. 10). The other striations seen in the *A. montereyensis* capsule micrographs (Fig. 8) are artifacts from damage to the knife edge by what appeared to be diatoms and small sand-like particles stuck to the jelly mass surrounding the capsules. In this particular species the capsules were not easily separable from the



Figs. 8-13. Transmission electron micrographs of capsule walls from five nudibranch species, all shown at the same final magnification. The outer surface of the capsule is towards the left for each figure, and the width of each capsule wall is demarcated with arrowheads. Fibrous material, believed to be part of the gelatinous egg mass, is seen on the outer capsule wall in Figures 8, 12 and (faintly) 13. **Fig. 8.** *Archidoris montereyensis,* about 5 d old, just prior to onset of embryonic movement. **Fig. 9.** *Cadlina laevis,* mid-veliger stage, age unknown. **Fig. 10.** *Hermissenda crassicornis,* age unknown, embryo shelled. **Figs. 11-12.** *Coryphella salmonacea,* 6 wk. and 4 wk. old veliger stages respectively, from different masses. **Fig. 13.** *Dendronotus frondosus,* fully shelled veliger stage, age unknown. A = granular material presumed to be albumen, present in the intracapsular space. Bar = 0.5 μm for all.

gelatinous egg mass, a portion of which is visible as scattered fibers on the outer capsule surface (Fig. 8, upper left). In other species, debris did not interfere with sectioning either because the capsules were easily separable from the gelatinous mass or because the jelly did not bind debris as readily.

Capsule morphology for each species was relatively consistent under the conditions used except for *Coryphella salmonacea*. In *C. salmonacea* the capsule wall in some sections was unlayered (Fig. 11); in other sections of capsules from a second mass the wall seemed layered, the outer part being of comparable width and texture but of greater electron density than the inner part (Fig. 12). Why the capsules of this one species sometimes but not always appeared layered is unclear. The influence of fixative contents, fixative osmotic concentration, and developmental stage on capsule morphology have yet to be determined.

Besides the fibrous material on the outer surface of some capsules (Figs. 8, 12, and 13), which is believed to be part of the gelatinous egg mass, some capsules of *Aeolidia papillosa* (Figs. 2, 7) and *Coryphella salmonacea* (Fig. 12) seemed to have projections on the inner capsule surface. However, the distinction between apparent capsule wall projections and intracapsular albuminous materials was often obscure. These projections did not appear to be a layer of vesicles as described by Clark and Goetzfried (1978) for a sacoglossan opisthobranch *Costasiella lilianae*. The inner capsule wall of other examined species was smooth.

Capsule wall thickness in the six species examined varied from a minimum of 0.07 μm in *Hermissenda crassicornis* (Fig. 10) to a maximum of 4.5 μm in *Archidoris montereyensis* (Fig. 8). Because apparent capsule wall thickness can vary with sectioning angle, the average observed thickness (not the maximum thickness resulting from oblique sectioning angle) was recorded (Table 2). Based on the few available data for the six species examined, capsule wall thickness was not obviously correlated with developmental type, days to hatching, or number of embryos per capsule (Table 2). There may be better correlations between characteristics of the gelatinous mass (thickness, durability) and developmental type or hatching time (Todd, 1981).

The thickest capsules occurred in members of the Doridacea but more species need to be examined to determine if dorids typically have thicker-walled capsules. Both thin walled and thick walled capsules surrounded embryos that would develop into planktotrophic larvae. For species with multiple embryos per capsule, more detailed study of capsule wall thickness is required to determine if capsule wall material stretches (is thinner) around larger groups of embryos or if a larger capsule of the same thickness is produced. The relationship between capsule wall thickness and prehatch developmental time is more problematical because hatching time is so temperature sensitive and because the six species examined were not reared at the same temperature (Table 2). Some species with shorter prehatch developmental periods had thinner capsules (e.g. *H. crassicornis*), yet one species with prolonged development (*C. laevis*) had a capsule of medium thickness and another species of medium hatching time had the thickest capsule wall (*A. montereyensis*).

SUMMARY

This paper reviews our knowledge of the origin, contents, adaptive value, composition, hatching, and structure of the embryonic capsules of nudibranch molluscs. Most comments in this paper probably also apply to other opisthobranch gastropods that produce small capsules within a gelatinous egg mass. Our knowledge is minimal and there are many areas of study left to be explored. We know the capsules are secreted by the parental reproductive system but it is unclear where and how the capsule wall and

Table 2. Comparison of embryonic capsule wall thickness with taxon, development type, approximate time to hatching, and number of eggs per capsule for six nudibranch species.

Species	Suborder	Development Type	Days to Hatching*	Eggs/ Capsule	Observed Capsule Wall Thickness
Archidoris montereyensis	Doridacea	Planktotrophic	20-24 @17°C[1] 23-28 @8-11°C	1-3	4.0–4.5 μm
Cadlina laevis	Doridacea	Non-planktonic Lecithotrophic	50 @10°C[1]	1	1.7–2.0 μm
Dendronotus frondosus	Dendronotacea	Planktonic Lecithotrophic	6 @14°C[2] 7-15 @8-11°C 32 @10°C[1]	1	0.25–0.35 μm
Aeolidia papillosa	Aeolidacea	Planktotrophic	10-24 @8-11°C	3-19	0.10–0.17 μm
Coryphella salmonacea	Aeolidacea	Non-planktonic Lecithotrophic	25-34 @5-8.5°C[3] 56 @5°C[4]	1	0.5-1.2 μm
Hermissenda crassicornis	Aeolidacea	Planktotrophic	7-8 @8-11°C 5-6 @ 13-15°C[5]	1-4	0.07–0.11 μm

*from Hurst, 1967, unless otherwise specified

[1]Thompson, 1967; [2]Williams, 1972; [3]Morse, 1971; [4]Eyster, 1985; [5]Harrigan and Alkon, 1978.

intracapsular fluid are secreted. Some species are known to have an intracapsular albuminous substance. The taxonomic distribution and chemical composition of this substance are still matters of debate. The capsules of nudibranchs are probably composed of some combination of carbohydrates and proteins, although proportions of carbohydrate to protein and actual composition may vary with species and even with time. The capsule walls are all thin, but vary in thickness from 0.1 to 4.5 μm in those species examined. Based on the few data available, capsule wall thickness is not obviously related to suborder, developmental type, hatching time, or number of embryos per capsule. The mechanisms by which nudibranch embryos manage to exit their capsules may include enzymatic, osmotic, and/or mechanical means, but all of these remain to be demonstrated. The proposed adaptive value of capsules and the surrounding gelatinous matrix is that they protect the developing embryos from infestation, predation, osmotic stress, desiccation stress, mechanical damage, and pollutant stress. Although some of these possible functions have been examined for prosobranch gastropods, none have been experimentally tested for opisthobranch gastropods.

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